NEW OBSERVATIONS ON THE ROYAL FERN HYBRID OSMUNDA × RUGGII

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The striking hybrid fern, *Osmunda* × *ruggii*, was described in 1940 by Rolla Tryon as the natural cross of *O. regalis* L. var. *spectabilis* (Willd.) Gray and *O. claytoniana* L. A single plant was discovered in Connecticut and grown in the garden of H. G. Rugg of Dartmouth College. Until the population described below was found, no new localities and no known occurrences of this taxon in nature have been reported. It is the only interspecific hybrid known in the Osmundaceae, a family conservatively interpreted as comprising three genera and 16 species (Bobrov, 1967, recognizes five genera and 36 species).

Because the fertile portion of the frond of full-sized hybrid plants exceeds in length those of either *Osmunda regalis* or *O. claytoniana*, F. S. Wagner (1974) suggested that its origin might involve *O. cinnamomea* rather than *O. claytoniana*. Our present interpretation is that the length of the fertile segment in *O.* × *ruggii* results from additive effects of *O. regalis* and *O. claytoniana*, and that *O. cinnamomea* is not involved in the parentage. This conclusion is based upon a number of new observations, herein reported, on *O.* × *ruggii* and its relatives, including data on habitat, morphology, cytology, and chemistry.

Habitat. We know practically nothing about the original collection site of the type specimen. There may have been two different collections, one at Wilton, the other near Hartford, Connecticut (Tryon, 1940). The hybrid proved to be a vigorous garden plant in Rugg's garden in Hanover, New Hampshire, and later in the gardens of Richard Harlow at LaAnna, Pennsylvania, of W. H. and F. S. Wagner at Ann Arbor, Michigan, and of others, where offsets were transplanted.

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For over a quarter of a century we have sought wild populations of this plant in connection with general studies of the role of hybridization in ferns. However, we were unsuccessful although we found the presumed parents growing together in hundreds of localities in the eastern United States and adjacent Canada. Finally, one of us (W. H. W.) encountered a very large colony in Craig County, Virginia, in July, 1974. The plants are scattered over an approximately elliptical area of 16.5 × 6.1 m. at the bottom of a ravine. The slope here is shallow and most of the plants are separated by a meter or more, although some of the crowns are only 10–20 cm. apart, suggesting recent separation. A total of 60 plants was found, so the colony is probably very old. The locality is in the Jefferson National Forest at an altitude of 765 m. on the south slope of Potts Mountain.

The forest type here is primarily montane oak-hickory with numerous ericaceous shrubs present, and the ferns grow in a mostly open understory, where a well developed canopy of tree crowns provides considerable shade. In general the soil is sandy and moist, and the ground is covered with leaf mold composed largely of rotting oak leaves. PH readings under plants of *Osmunda* × *ruggii* gave 6.5.

The forest canopy is dominated by *Acer rubrum*, *Liriodendron tulipifera*, and *Quercus primus*. Other woody plants in the habitat include species of *Betula*, *Castanea*, *Carya*, *Cornus*, *Fraxinus*, *Lindera*, *Oxydendron*, *Quercus*, *Parthenocissus*, *Robinia*, *Rhododendron*, *Rubus*, *Sassafras*, *Smilax*, *Toxicodendron*, and *Vitis*. The understory is dominated by *Osmunda cinnamomea* L., *O. claytoniana*, and *O. regalis*, which are roughly equal in abundance. The lady-fern, *Athyrium filix-femina*, is also common here. Other herbaceous plants include members of the genera *Amphicarpa*, *Asplenium*, *Botrychium*, *Collinsonia*, *Chimaphila*, *Danthonia*, *Dioscorea*, *Galax*, *Goodyera*, *Habenaria*, *Lobelia*, *Hypericum*, *Panicum*, *Polystichum*, *Scutellaria*, *Smilacina*, *Solidago*, *Thelypteris*, *Veronica*, and *Viola*.

Assuming that the population of *Osmunda* × *ruggii* in the Potts Mountain ravine represents a single clone, it may be the result of hybridization that occurred many centuries ago. In New England, Steeves and Wetmore (1953) estimated that an average annual in-

Figure 1. Frond of *Osmunda* × *ruggii* in middle of photograph with *O. regalis* in the upper background. The photograph was taken at the Virginia locality.

crease in rhizome length in *O. cinnamomea* may be only several millimeters. Klekowski and Berger (1976) gave an approximate growth rate of 0.7 cm. per year for rhizomes of *O. regalis*. Assuming that growth began in the center of the population, and using the latter estimated rate of increase, the age of the *O.* × *ruggii* colony would be greater than 1100 years.

Living plants from Potts Mountain have been introduced at U. S. National Arboretum in Washington, D. C. There is no reason why the fine wild population of *Osmunda* × *ruggii* should not continue to flourish in the future as there is no known threat from natural causes, the habitat is remote, and the District Rangers of the Jefferson National Forest are aware of the interesting nature of the colony and plan to protect its habitat from lumbering operations and vandalism.

Morphology. Growing side-by-side in their native habitat, the four taxa of *Osmunda* are readily distinguishable from distances up to 5–10 m. *Osmunda cinnamomea* differs from the others in July and August in its spreading fronds which are light yellow-green in color and more glossy. Its pinna segments are more conspicuously pointed. *Osmunda regalis* is obvious because of its frond structure, the pinnae with widely separated, large and rounded pinnules and the fronds overlapping each other in dense intergrowth. *Osmunda claytoniana* displays the strictest habit, the fronds of mature individuals nearly upright and parallel. The laminar luster is dull and the color bluish-green. In over-all habit, *O.* × *ruggii* is closest to *O. claytoniana*. The hybrid is like *O. claytoniana* in general shape and appearance, but the pinnae are divided as in *O. regalis* (Figure 1).

For each *Osmunda* taxon in the valley we counted the number of fronds on each of 20 crowns. The average and range for *O. cinnamomea* was 5.3 (3–9), for *O. regalis* 6.8 (4–10), and for *O. claytoniana* 10.0 (6–17). For the hybrid, surprisingly, these values were only 5.0 (3–9). A reason for the absence of intermediacy in frond number per rhizome is unknown. Perhaps it is a peculiarity of this specific genotype and not significant because all of the plants involved, species and hybrid, represent as few as four genetically unique clones.

A disappointing feature of the Potts Mountain *Osmunda* × *ruggii* colony was the total lack of fertile fronds at the time of our

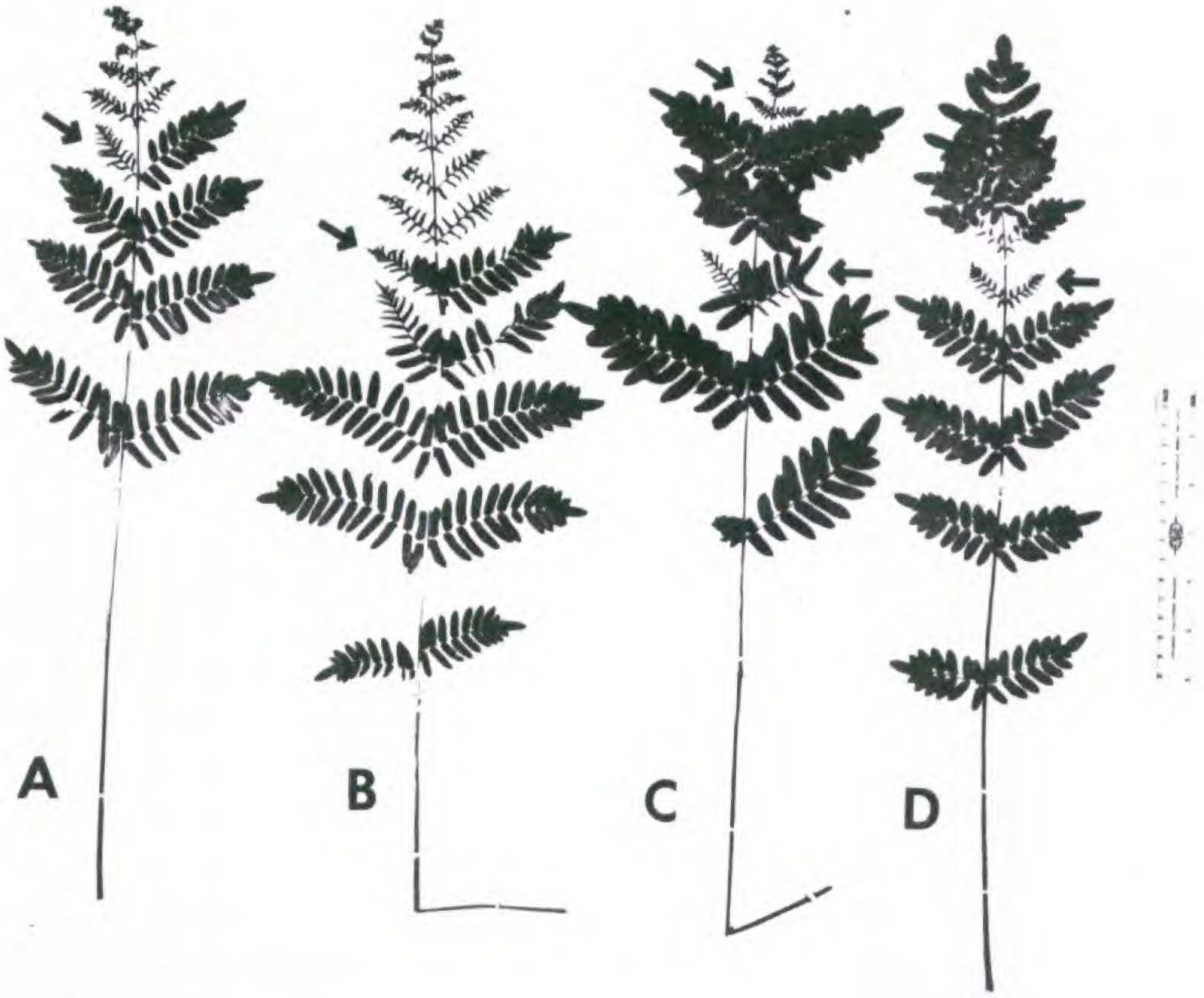

Figure 2 *Osmunda* × *ruggii* fronds transplanted from the Virginia locality to Ann Arbor, with apical (A, B), medial (D), and combined apical and medial fertile pinnae (C).

study in July, 1974. Accordingly, we asked Gerald B. Straley of Eggleston, Virginia, to revisit the spot in the middle of May, 1975, and examine the *Osmunda* populations. He reported as follows: "The *O. claytoniana* fertile fronds were completely developed and the fertile pinnae dark green. The *O. regalis*, on the other hand, had fertile fronds still unfurling and many of the fertile pinnae were still inrolled. There was not one fertile frond to be found on *O. ruggii* and I searched every plant closely."

Fortunately, plants which were transferred to Washington, D. C., and Ann Arbor, Michigan, produced fertile fronds, presumably as a result of transplanting. The fertile fronds displayed beautiful irregularity of expression of pinna dimorphism. In some (Figure 2,

A, B) the fertile pinnae were apical, as in *Osmunda regalis*. In others (Figure 2, D) the fertile pinnae were medial, as in *O. claytoniana*. Most interesting were those that showed both conditions on the same frond (Figure 2, C). It should be noted that all of these fronds are fairly small.

The much larger fertile fronds of the cultivated plants growing in the Richard Harlow garden in Pennsylvania show a uniform structure quite different from that in the transplants from the Virginia colony as well as from both parents. On 22 June, 1973, we studied several distinct colonies at the Harlow garden. Among the dozens of fertile fronds observed, all displayed the terminal condition of the fertile pinnae (Figure 3). However, these extended nearly the entire distance to the base of the blade, there being only one to three pairs of sterile pinnae, the remaining 10–15 pinnae being fertile. Indeed, with only a slight change, the conversion of the bottom pinnae into the fertile condition, we would have the situation familiar in the wholly modified sporophyll of *Osmunda cinnamomea*. So unexpected was this condition, in fact, that we wondered whether it was possible that the original Rugg material, from which the Harlow garden specimens were derived, may not have been *O. cinnamomea* × *regalis* rather than *O. claytoniana* × *regalis*. A more likely interpretation of the unusually long fertile segment is that it displays the additive effects of the terminal fertile pinnae of *O. regalis* plus the medial ones of *O. claytoniana*. An explanation such as this would be of special interest as a rare case in which a hybrid fern is not strictly intermediate in all its characters.

In most other respects, however, the hybrid is intermediate between *Osmunda claytoniana* and *O. regalis*. The segments are intermediate in size, although slightly closer to *O. claytoniana* than to *O. regalis*. The segment bases are of special significance in that the parental ones are strongly differentiated. Those of *O.* × *ruggii* are narrowly adnate, whereas those of *O. regalis* are obviously petiolulate in contrast to those of *O. claytoniana*, which are so strongly adnate as to be connate at their bases with the adjacent segments. In the hybrid the segment bases (especially the proximal ones) are sometimes so strongly contracted as to produce a small stalk. The finely dentate margin of *O. regalis* is barely expressed in *O.* × *ruggii*, which is nearly entire, as in *O. claytoniana*.

As R. Tryon (1940) noted, most of the veins of *Osmunda* × *ruggii* segments fork only once, as in *O. claytoniana*, rather than twice,

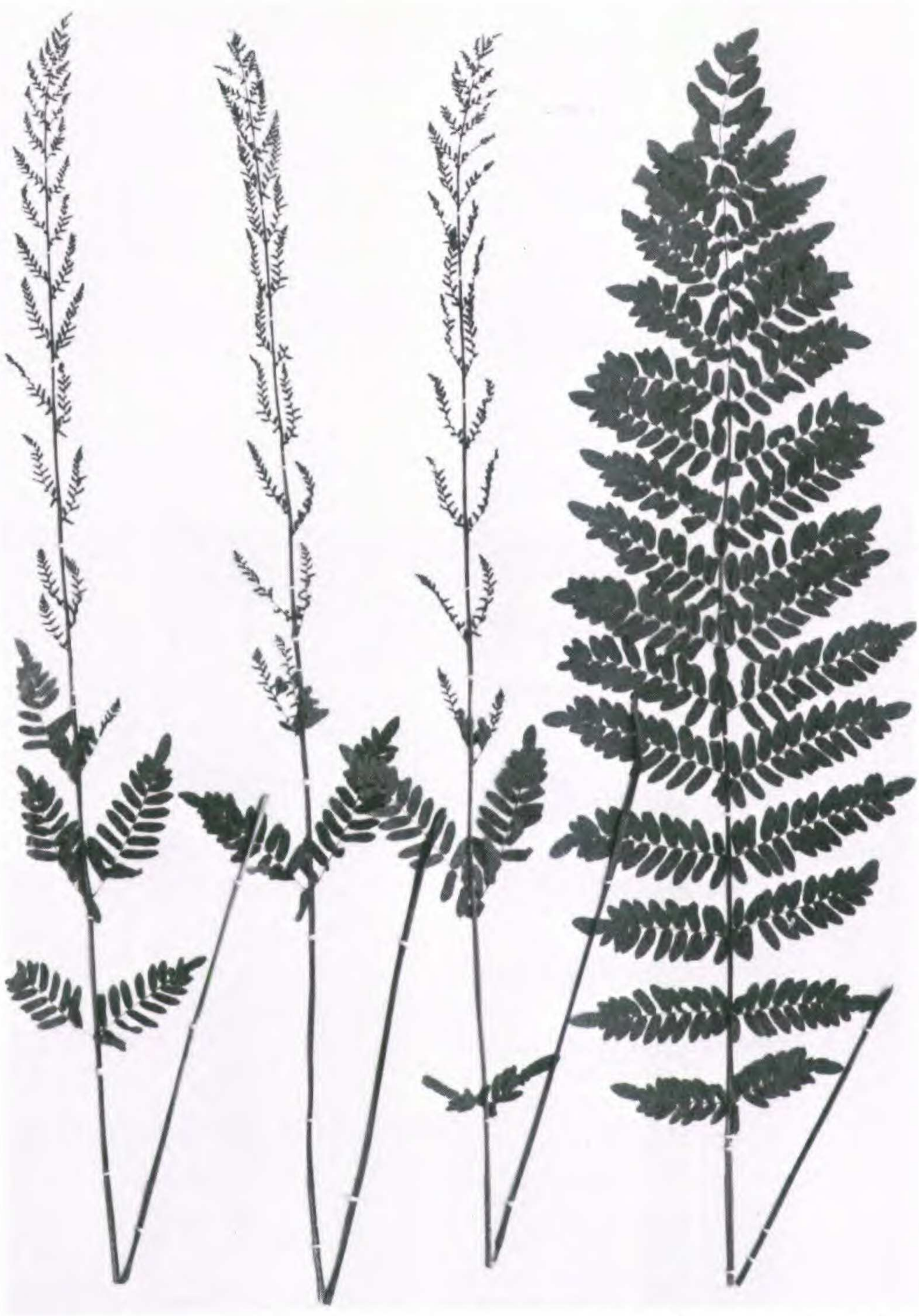

Figure 3. *Osmunda* × *ruggii* full-sized fronds from the Harlow garden in Pennsylvania with unexpectedly long terminal fertile segments; sterile frond at right.

as in *O. regalis* (Figure 5). The positions of the furcations have not previously been contrasted: the branching of the veins in *O. claytoniana* occurs one-fifth to one-third of the distance to the vein tips. In *O. regalis,* most of the veins branch at the base or even in the costa so that the branches come out separately. *Osmunda* × *ruggii* is intermediate in this respect, the branchings occurring anywhere from the base to approximately one-fifth of the distance to the vein tips.

Anatomy. Anatomical features of the species involved in this study have been described by Miller (1967, 1971) and Hewitson (1962). The most significant comparisons of the species with the hybrid involved leaf trace branchings, root number, endodermis, and distribution of sclerenchyma in the stem cortex and leaf base. The first branchings of the leaf trace protoxylem occur in the leaf bases in *Osmunda cinnamomea* and *O. claytoniana.* In *O. regalis* branching is in the inner cortex. Using materials from the Harlow garden, Miller found that *O.* × *ruggii* shows an intermediate branching position in the outer cortex. The number of roots associated with a single leaf trace in *O. cinnamomea* is generally only one, but in *O. claytoniana, O.* × *ruggii,* and *O. regalis,* this number is generally two.

Another distinction of *Osmunda cinnamomea* from the other three taxa is the presence of an internal endodermis. This tissue is unknown in any other recent species of the family Osmundaceae. *Osmunda claytoniana, O.* × *ruggii,* and *O. regalis* conform to the usual condition in the living members of the family in having only an external endodermis. Likewise, *O. cinnamomea* is peculiar in having a nest of thick-walled fibers in the inner cortex of the rhizome adaxial to each leaf trace. Such nests are known only in this species and in *Todea barbara* among extant members of the family. There are no such clusters of fibers present in *O. claytoniana, O.* × *ruggii,* and *O. regalis.* In addition, *O. cinnamomea* is unique in having three groups of thick-walled fibers, one on each side and one abaxial in the outer cortex (sclerenchyma ring) of the leaf base. In *O. claytoniana, O.* × *ruggii,* and *O. regalis* leaf bases the thick-walled fibers form an arch that occupies most of the abaxial semicircle of the sclerenchyma ring.

Thus, of the distinctive anatomical features of *Osmunda cinnamomea,* only one — the first branching of the leaf trace protoxylem

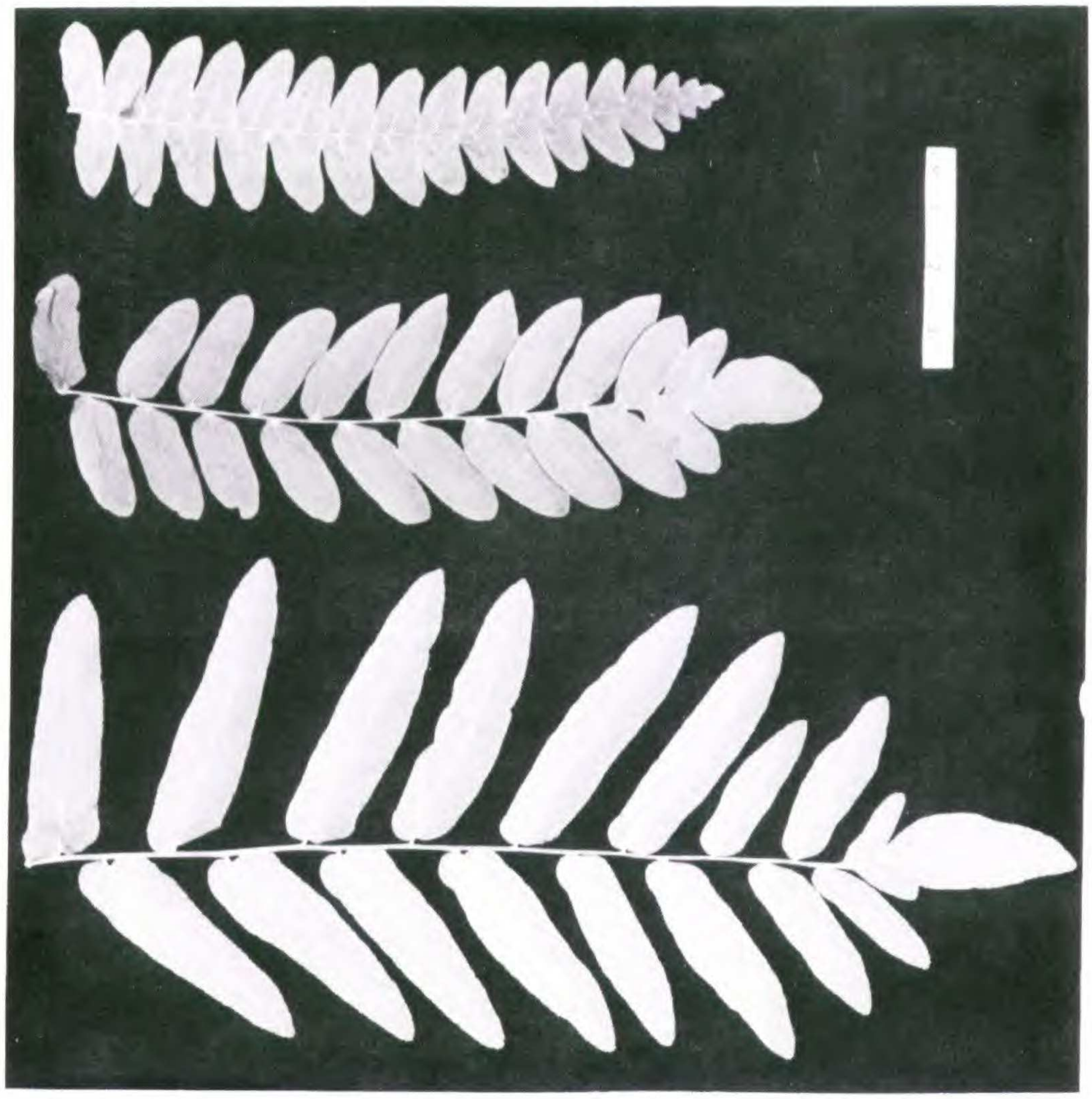

Figure 4. Pinnae of *Osmunda*. Upper, *O. claytoniana*. Middle, *O.* × *ruggii*. Lower, *O. regalis*.

— shows evidence of expression in *O.* × *ruggii*. However, since *O. claytoniana* shares this character with *O. cinnamomea*, it has no diagnostic value. All of the anatomical characters of *O.* × *ruggii* are consistent with the original hypothesis of its origin.

Chemistry. Specimens of all four taxa were examined chromatographically by D. H. Wagner for phenolics. Several pinnae from each specimen (vouchers deposited in Marion Ownbey Herbarium, Washington State U., Pullman) were ground in a mortar with grinding sand. The samples were extracted separately with methanol and two-dimensional paper chromatograms prepared according to standard procedures (Mabry, et al., 1970). BAW (4:1:5 n-butanol,

gla. acetic acid, water) was used in the first direction and 15% acetic acid for the second direction. Although it was evident that there was no variation in chromatographic patterns within each of the taxa, comparison among them was difficult due to streaking and poor separation of the compounds caused by non-phenolic substances present in the extracts. The extracts from each taxon were pooled and phenolics separated by precipitating with lead (II) acetate (procedure described in D. Wagner, 1976). Chromatograms prepared from the purified extracts are exceptionally clear. Comparison with the first set of chromatograms indicated that few, if any, compounds were lost by the purification.

Diagrams of the chromatograms produced from the pooled extracts are shown in Figure 6. A total of twenty-one compounds can be detected on the chromatograms of *Osmunda* × *ruggii*. These fall into three categories — those shared with none of the species, those shared with only one of the species, and those shared with two of the species.

Four of the compounds in *Osmunda* × *ruggii* can be clearly identified with spots on the chromatograms of any of the three species. Three are shared with both *O. regalis* and *O. claytoniana*. Three are shared with both *O. claytoniana* and *O. cinnamomea*. One is shared with both *O. regalis* and *O. cinnamomea*.

Those compounds of *Osmunda* × *ruggii* that are shared with only one of the species provide evidence regarding origin of the hybrid. Five compounds extracted from *O.* × *ruggii* are shared only with *O. regalis* (Figure 6, ru & re hatchings to the right). Five compounds are shared only with *O. claytoniana* (Figure 6, ru & cl hatching to the left). None of the compounds found in *O.* × *ruggii* is shared only with *O. cinnamomea*. These results support Tryon's original interpretation.

Cytology. Fertile pinnae of a transplant of *Osmunda* × *ruggii* from the Harlow garden were collected during the last two weeks of April, 1973, in Ann Arbor, Michigan. They were fixed in Newcomer's solution after cold pretreatment with paradichloro-benzene aqueous solution. Squashes were made in a mixture of 50% aceto-carmine and 50% Hoyer's solution. The photographs (Figure 7) were made at 500× using Zeiss phase contrast equipment.

We anticipated that meiosis would be abnormal, because abortion of the spores is so extreme. Indeed, we found it impossible

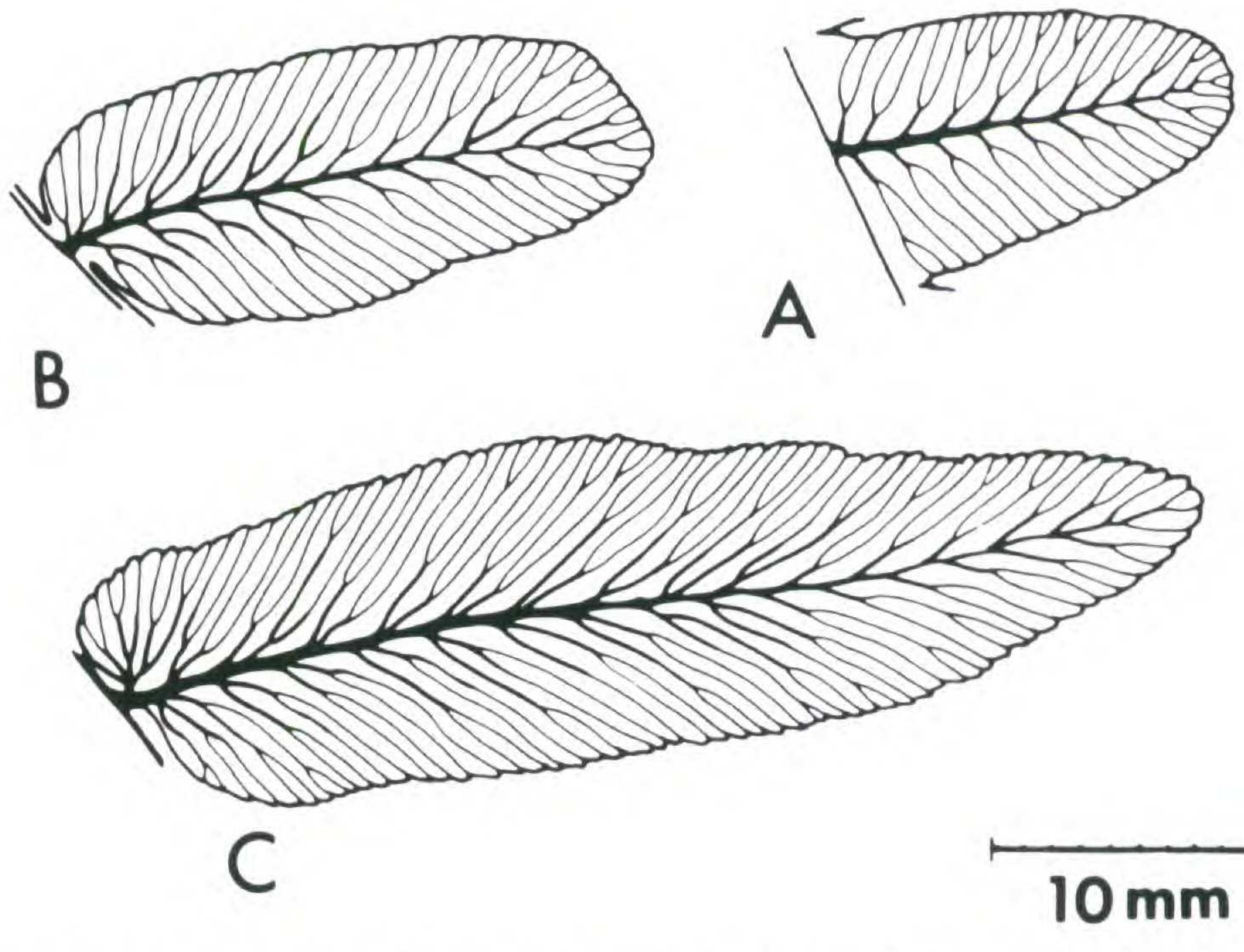

Figure 5. Venation patterns in *Osmunda* pinnae. A, *O. claytoniana*. B, *O.* × *ruggii*. C, *O. regalis*.

to observe individual spores in the sporangia. The capsules contain coalesced masses of thin-walled cells. The sporangial walls themselves do not form normally either, the walls being somewhat fleshy.

In striking contrast to the very uniform conditions thus far reported in the Osmundaceae, with 22 pairs at meiotic metaphase, *Osmunda* × *ruggii* shows 44 units, each unit a single chromosome. The univalents appear as short rods. We found no tendency at all for bivalent groupings, indicating a total lack of pairing factors prior to anaphase. The preliminary report of cytological conditions in *O.* × *ruggii* (F. S. Wagner, 1974) is based on garden specimens derived from the original Rugg plant. Later studies in 1975 of garden transplants from the Potts Mountain colony revealed the same lack of pairing of 44 chromosomes.

Significance. New evidence from a wide variety of sources overwhelmingly supports R. Tryon's hypothesis that *Osmunda* × *ruggii* is the natural hybrid of *O. claytoniana* and *O. regalis*. In spite of the *O. cinnamomea*-like fertile fronds of the large plants of the hybrid growing in the Harlow garden at LaAnna, Pennsylvania,

there is little support for regarding *O. cinnamomea* as a possible parent. Most of the fertile fronds from smaller plants of *O.* × *ruggii* show more intermediate conditions, including not only terminal fertile pinnae, but medial, and even both on the same frond.

Osmunda × *ruggii* is now known in a natural population from only one locality in spite of the fact that its parents occur together over a huge area from northeastern Canada to the Appalachians in the south and Minnesota in the west, probably the best explored area botanically in the New World. At maturity the appearance of *O.* × *ruggii* is so splendid and distinctive that an experienced field botanist would recognize it immediately. We can only speculate on causes for its rarity. Perhaps it cannot compete with other plants in its native habitats. At the Potts Mountain locality in Virginia, however, it shows no evidence of inability to compete. Perhaps too, there are strong incompatibility barriers between the parents, and only very rare mutations can hybridize. Experimental investigations in the laboratory may provide some clues.

The hybrid origin of *Osmunda* × *ruggii* bears upon the subgeneric classification of the Osmundaceae. In over-all appearance, *O. claytoniana* and *O. cinnamomea* are so similar that sterile fronds are commonly confused by field workers. *Osmunda regalis,* on the other hand, is very distinctive. Thus, a hybrid between either *O. claytoniana* or *O. cinnamomea* and *O. regalis* would seem to be most unusual. It would be more logical to expect a hybrid between *O. claytoniana* and *O. cinnamomea.*

Of recent taxonomic treatments, those of Bobrov (1967) and Hewitson (1962) place *Osmunda cinnamomea* and *O. claytoniana* together in subg. Osmundastrum, separating them from *O. regalis* in the type subg. Osmunda. In contrast, Miller (1971), although recognizing the two subgenera, places *O. claytoniana* with *O. regalis* in subg. Osmunda. Miller's conclusions are based upon comparative anatomy of fossil and living forms, and they seem to be in conflict with more recent conclusions based upon comparative chemistry. A serological and disc electrophoretic analysis of spore proteins (Petersen & Fairbrothers, 1971) showed that *O. cinnamomea* and *O. claytoniana* have greater protein affinities for each other than either has for *O. regalis.* DNA hybridization techniques were used to estimate the degree of DNA base sequence homology between the three species. Stein and Thompson (1975) concluded that under conditions of extensive reassociation, measurements of

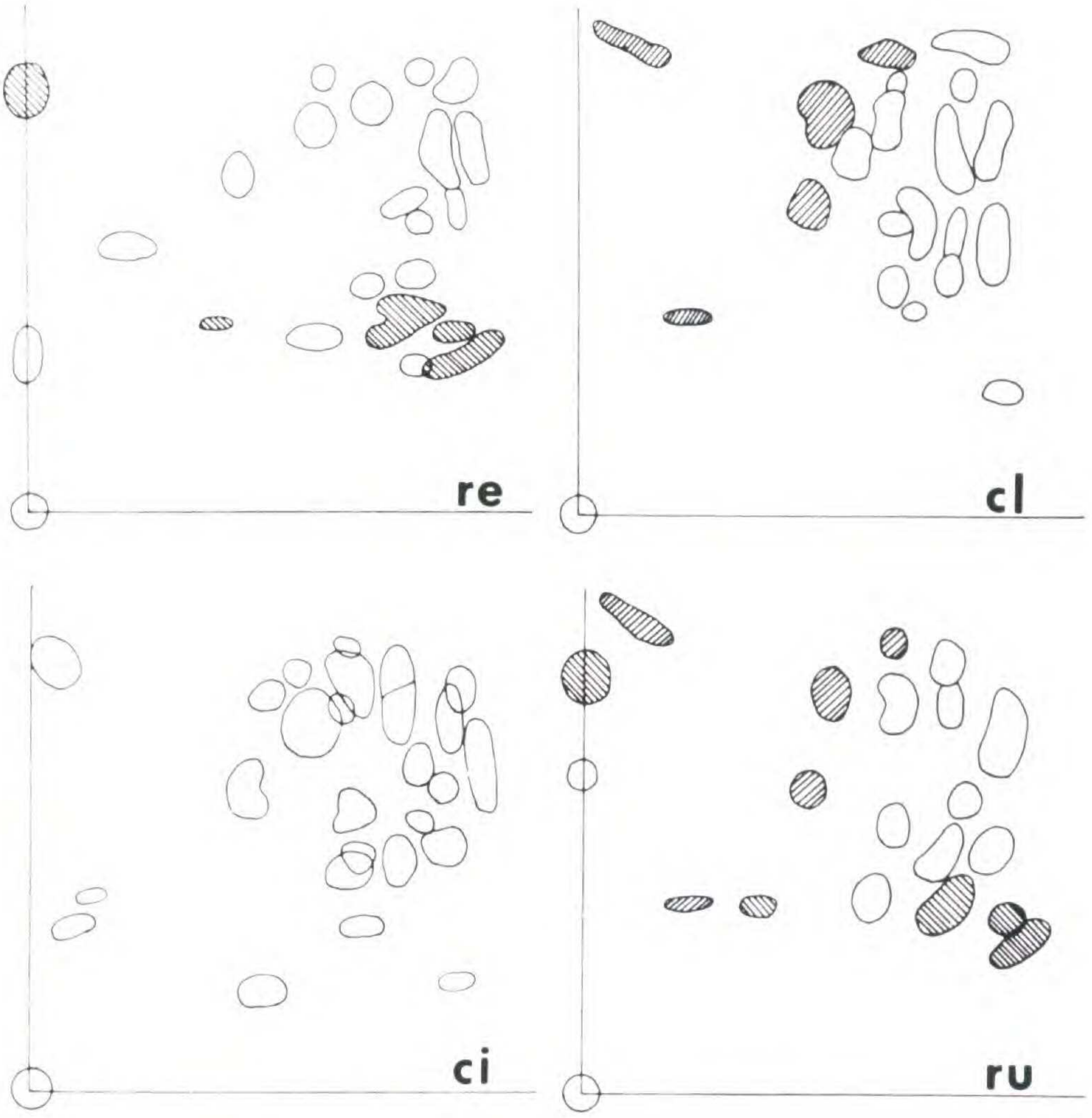

Figure 6. Diagrams of chromatograms of *Osmunda*. Vertical axis = BAW; horizontal axis = 15% acetic acid. Letters refer to specific epithets.

interspecific reaction and thermal stability of hybrid molecules indicated that *O. claytoniana* shares more DNA homology with *O. cinnamomea* than it does with *O. regalis*. The conclusion of both of these chemical studies is that *O. claytoniana* and *O. cinnamomea* should be placed together in subg. Osmundastrum, although Petersen and Fairbrothers (op. cit.) do state that *O. regalis* has, in general, greater protein affinities for *O. claytoniana* than it has for *O. cinnamomea*.

As has been demonstrated again by the study of *Osmunda* × *ruggii*, there is no question that *O. claytoniana* and *O. regalis* are more closely related anatomically to each other than either is to

O. cinnamomea, reaffirming Miller's earlier findings. Indeed, morphologically it is only in characters of leaf cutting and segment structure that *O. claytoniana* and *O. regalis* differ. Although spore protein studies might be difficult to carry out with *O.* × *ruggii* because of the extreme spore abortion, it would be interesting to repeat the DNA hybridization experiments using *O.* × *ruggii* together with its parents and with *O. cinnamomea.*

Hickok and Klekowski (1975) have discussed the possible significance of the absence of chromosome pairing in *Osmunda* × *ruggii.* It has been hypothesized by Klekowski (1973) that the genus *Osmunda* possesses duplicated sets of four chromosomes, but that pairing is restricted to bivalent formation through some sort of physiological control. Lack of structural divergence within the homoeologous sets could be explained through the prevention of structural diploidization by occasional homoeologous pairing within the sets. Accordingly, one might expect in an interspecific hybrid that there would be a level of autosyndetic pairing. The fact that we found no pairing at all, only univalents, in *O.* × *ruggii,* together with the fact that synthetic autopolyploids experimentally produced by Irene Manton (1950) displayed multivalent formation, creates a puzzling situation and casts doubt on Klekowski's hypothesis.

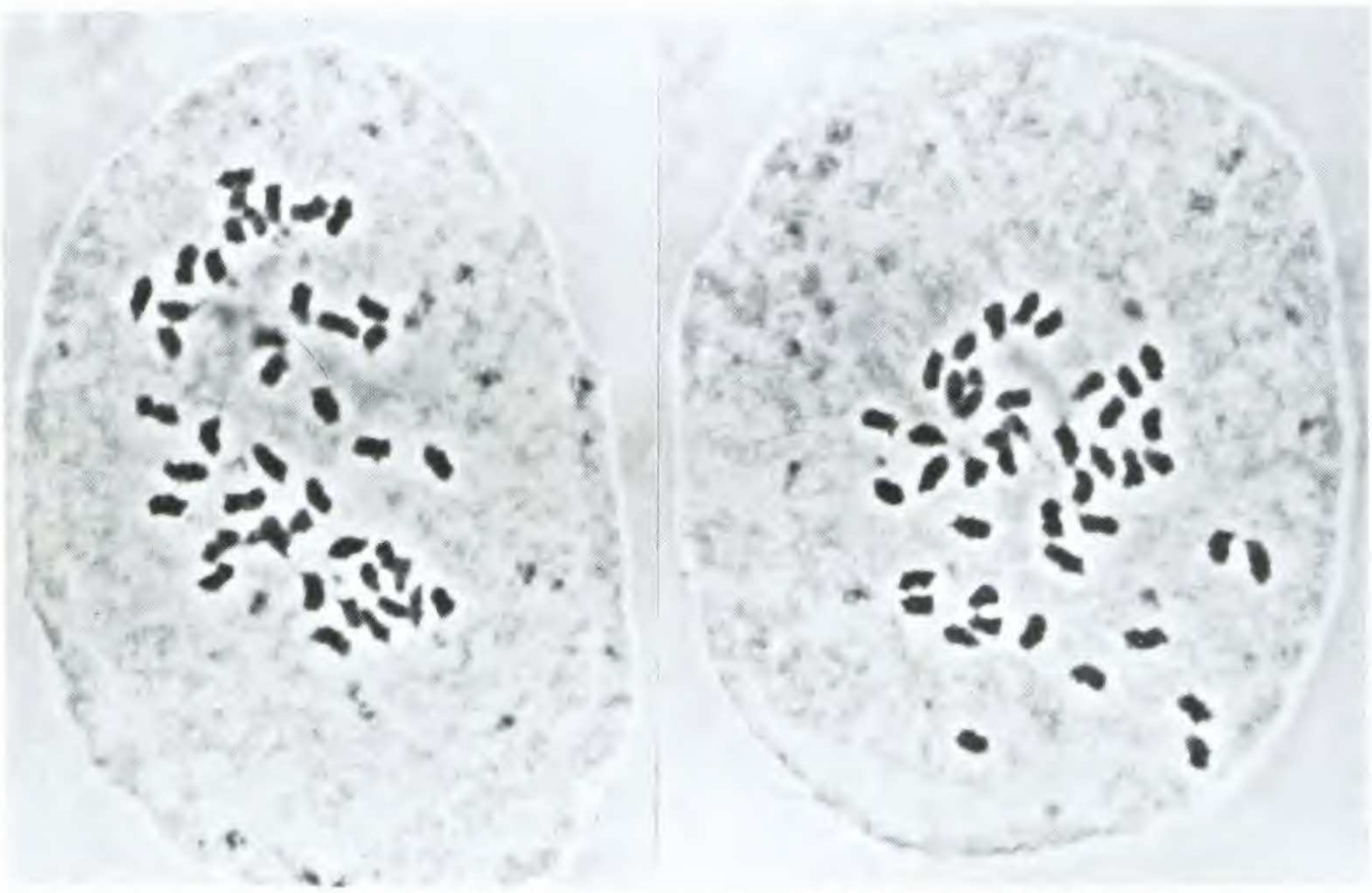

Figure 7. Meiosis in *Osmunda* × *ruggii* with 44 unpaired chromosomes.

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